

Serodiagnosis of Respiratory Syncytial Virus (RSV) Infection in Children as Measured by Detection of RSV-Specific Immunoglobulins G, M, and A with Enzyme-Linked Immunosorbent Assay

MARC J. M. MEDDENS,[†] PAUL HERBRINK,* JAN LINDEMAN, AND WILLEMEN C. VAN DIJK

Department of Pathology, Clinical Microbiology and Clinical Immunology, Diagnostic Centre, Stichting Samenwerking Delftse Ziekenhuizen, P. O. Box 5010, 2600GA Delft, The Netherlands

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The diagnostic value of an enzyme-linked immunosorbent assay for detection of respiratory syncytial virus (RSV)-specific immunoglobulin G (IgG), IgM, and IgA in sera from infants and children with proven RSV infection, from a control group, and from patients with symptoms of viral respiratory disease was analyzed. Compared to virus isolation and RSV antigen detection methods, the sensitivity of this assay was 87% and the specificity was 79%. For IgG alone, these were 45 and 92%, for IgM alone they were 48 and 92%, and for IgA alone they were 74 and 95%, respectively.

Respiratory syncytial virus (RSV) infection can be diagnosed rapidly by direct detection of RSV antigens in nasopharyngeal secretions (NPS), and results can be available within 1 h (1-3). This rapid method is specific and at least as sensitive as isolation. In this paper, an enzyme-linked immunosorbent assay (ELISA) for the diagnosis of RSV infection by detection of class-specific antibodies in paired serum samples is described. Its diagnostic value in relation to RSV isolation and direct detection of RSV antigens is assessed.

Three groups of patients were divided into two age groups: those ≤ 1 year old (infants) and those 1 to 7 years old (children). Serum samples were drawn in the acute phase of the disease and 10 to 14 days later.

Control group. The control group consisted of patients clinically suspected of having an infection. Two serum samples were obtained from each of 8 infants and 18 children in the summer. In three patients, viral infections were diagnosed: one case of measles, one case of adenovirus infection, and one case of cytomegalovirus infection.

Proven RSV infection group. The RSV patient group included patients with RSV infection proven by RSV isolation or antigen detection (31 patients) or by a fourfold increase in titer in RSV complement fixation (CF) (2 patients). Paired serum samples and NPS were obtained from each of 16 infants and 15 children.

Patients without a proven RSV infection. Patients with symptoms of viral respiratory disease but negative by RSV isolation and RSV antigen detection and negative for a fourfold increase in titer in RSV CF in paired serum samples composed the third group. Both paired serum samples and NPS were obtained from each of 12 infants and 26 children; paired serum samples were obtained only from 5 infants and 15 children.

ELISA was performed essentially as described previously for *Campylobacter jejuni* (4), except that whole-cell sonic extracts of RSV-infected HEP-2 cells and of uninfected cells were used as RSV antigen and control antigen, respectively

(RSV-Ag and Co-Ag), and *ortho*-phenylenediamine was used as the substrate. Serum samples positive for RSV-specific immunoglobulin M (IgM) or IgA were assayed for rheumatoid factor by ELISA (15). IgM and IgA fractions prepared by fast-protein liquid chromatography (11) were taken from serum samples positive for IgM or IgA rheumatoid factor and retested in the RSV ELISA.

RSV was isolated by standard procedures. RSV antigen detection was performed with direct immunofluorescence (Imagen, Boots, Birkshire, United Kingdom). Differences in incidence between groups were analyzed with the chi-square test, differences between CF and ELISA results were analyzed with the McNemar test, and differences in the absorbance (ΔA) ($=A[\text{RSV-Ag}] - A[\text{Co-Ag}]$) between serum sample pairs in patient groups were analyzed by the one-tailed paired Student's *t* test.

Detection of RSV-specific IgG. Detection of RSV-specific IgG is shown in Fig. 1a. Only in the RSV-infected children was the median anti-RSV IgG titer of the second serum sample significantly higher than that of the first (1:12,800 versus 1:800, $P < 0.01$). Fourfold or greater increases in IgG titer were found in 14 of the 31 patients, whereas in the control group no fourfold or greater increases were found.

Detection of RSV-specific IgM. Detection of RSV-specific IgM is shown in Fig. 1b. Only in the RSV-infected infants was the mean ΔA_{IgM} of the second serum sample significantly higher than that of the first (0.41 ± 0.83 versus 0.08 ± 0.12 , $P < 0.01$). An increase of at least 0.2 absorbance units in paired serum samples were measured in 15 of the 31 RSV patients. In the control group, no such increases were measured, indicating that a ΔA_{IgM} increase of 0.2 absorbance units in a pair of serum samples could be used as the second criterion for diagnosis of recent RSV infection.

Detection of RSV-specific IgA. Detection of RSV-specific IgA is shown in Fig. 1c. The mean ΔA_{IgA} was significantly higher in the second serum sample than in the first one for the RSV patients in both age groups ($P < 0.01$). A ΔA_{IgA} increase of at least 0.2 absorbance units was measured in 23 of the 31 RSV patients, whereas no such increase was found in the control group. This increase is used as the third criterion for diagnosis of a recent RSV infection. To evaluate

* Corresponding author.

[†] Present address: Eurodiagnostics, 7303GC Apeldoorn, The Netherlands.

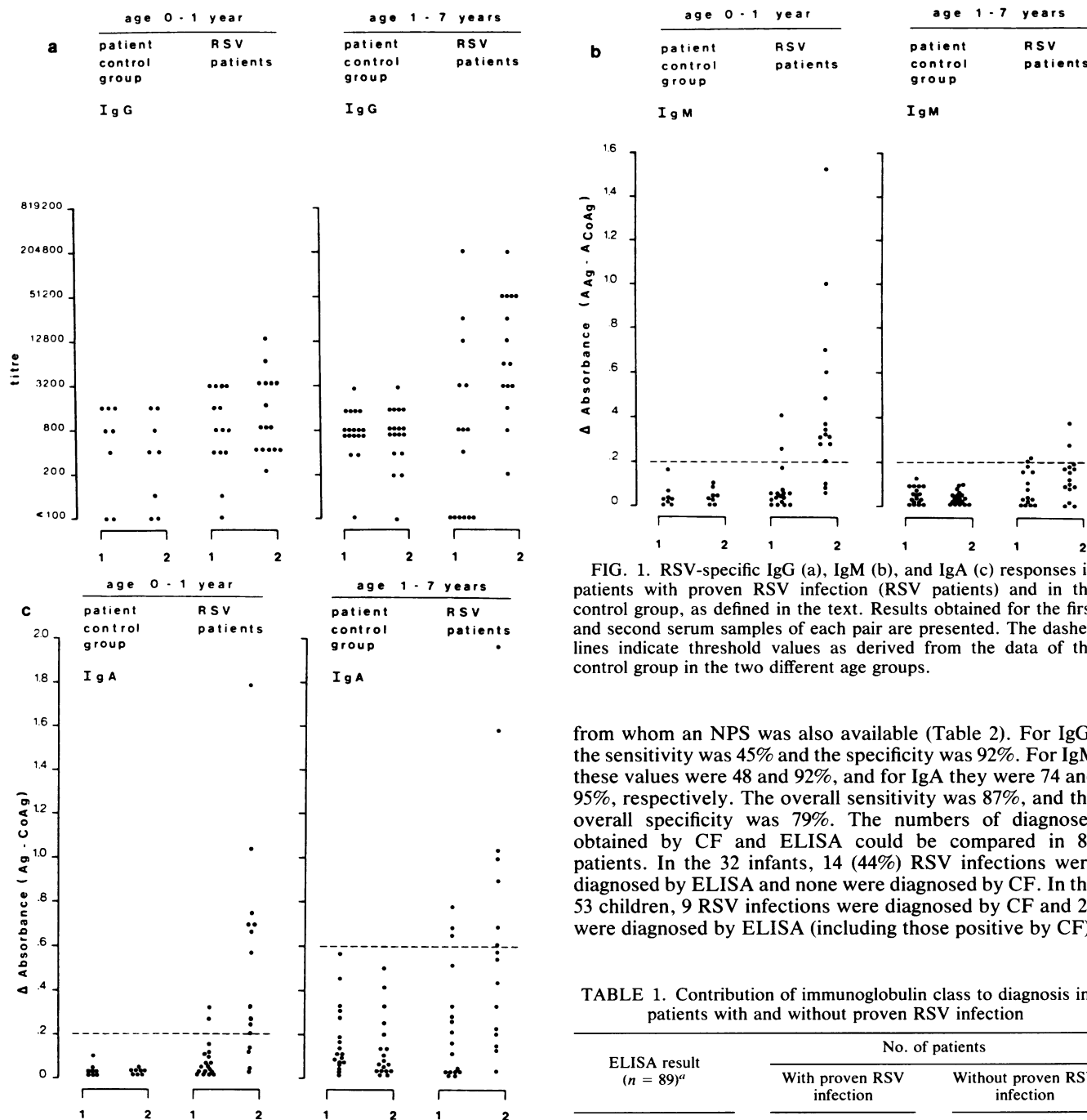


FIG. 1. RSV-specific IgG (a), IgM (b), and IgA (c) responses in patients with proven RSV infection (RSV patients) and in the control group, as defined in the text. Results obtained for the first and second serum samples of each pair are presented. The dashed lines indicate threshold values as derived from the data of the control group in the two different age groups.

from whom an NPS was also available (Table 2). For IgG, the sensitivity was 45% and the specificity was 92%. For IgM these values were 48 and 92%, and for IgA they were 74 and 95%, respectively. The overall sensitivity was 87%, and the overall specificity was 79%. The numbers of diagnoses obtained by CF and ELISA could be compared in 85 patients. In the 32 infants, 14 (44%) RSV infections were diagnosed by ELISA and none were diagnosed by CF. In the 53 children, 9 RSV infections were diagnosed by CF and 25 were diagnosed by ELISA (including those positive by CF).

TABLE 1. Contribution of immunoglobulin class to diagnosis in patients with and without proven RSV infection

ELISA result (n = 89) ^a			No. of patients			
			With proven RSV infection		Without proven RSV infection	
IgG rise	IgM rise	IgA rise	Infants 0-1 yr	Children 1-7 yr	Infants 0-1 yr	Children 1-7 yr
+	+	+	3	2	0	0
-	+	+	8	1	0	1
+	-	+	0	6	0	0
+	+	-	0	0	0	1
+	-	-	0	3	0	2
-	+	-	1	0	1	2
-	-	+	2	1	0	7
-	-	-	2	2	16	28

^a For the respective immunoglobulin class an immune response in the paired serum samples was measured; -, there was no immune response measured. The criteria for a positive result are given in the text.

the diagnostic usefulness of the ELISA, we analyzed paired serum samples from the 58 patients in whom an RSV infection could not be detected by virus isolation, RSV antigen detection, or a fourfold increase in titer in RSV CF (Table 1). In these patients, an immune response to RSV was found by ELISA in 1 of the 17 infants and in 13 of the 41 children. This is an increase in the total diagnostic yield of 22% (from 65 to 79 diagnoses).

The sensitivity and specificity of the ELISA in relation to RSV isolation and antigen detection methods was investigated by using the serum sample pairs from the 69 patients

TABLE 2. Comparison of ELISA with RSV isolation and antigen detection

RSV isolated and/or RSV antigen detected (<i>n</i> = 69) ^a	No. of patients diagnosed by ELISA with ^b :							
	IgG rise		IgM rise		IgA rise		Combined IgG, IgM, and IgA rise	
	Yes	No	Yes	No	Yes	No	Yes	No
Yes	14	17	15	16	23	8	27	4
No	3	35	3	35	2	36	8	30

^a Patients with (yes) and without (no) proven RSV infection on whom ELISA and RSV isolation/antigen detection were performed.

^b Criteria for diagnosis of RSV infection are described in the text.

The overall incidence of an anti-RSV IgG response of the 41 patients in whom an RSV infection could be diagnosed by ELISA was significantly lower in the infants than in the children (3/15 versus 15/26; chi-square = 5.49, $P < 0.02$). Meurman et al. (6) found IgG responses in 24 of the 26 patients under 2 years of age who had a proven primary RSV infection. The titers reached maximum levels between 20 and 30 days after the onset of illness. To detect a significant increase in titer in RSV-infected infants under 6 months of age, Nandapalan (9) had to start with serum dilutions as low as 1:4 in several cases. Hornsleth et al. (5) showed that in infants and small children during a primary RSV infection, only IgG1 and IgG3 antibodies can be detected. Wagner et al. (12) showed that in infants and young children between 4 and 21 months of age the IgG immune response was mainly of the IgG1 and IgG3 subclasses. All 20 patients studied showed an IgG1 immune response against the F glycoprotein, whereas 19 patients showed an immune response of the IgG3 subclass. Watt et al. (13) reported a marked increase in both IgG1 and IgG3 in most infants over 6 months of age. However, in infants under 6 months of age an IgG immune response could not be detected, although increases in levels of IgA and IgM were observed. Murphy et al. (7, 8), by using an ELISA with a total IgG conjugate, also reported a lower incidence in IgG response in infants than in children.

These discrepancies in the IgG immune response in RSV infection might be explained at least in part by the presence of maternal antibodies in very young infants, antibodies which possibly mask the IgG immune response when measured with total-IgG-specific conjugate. Since IgG3 has half-life kinetics similar to those of IgM, masking of the IgG immune response is not likely to occur when IgG3-specific conjugates are used, resulting in a higher incidence of IgG immune response as was measured by Wagner et al. (12).

A significant ΔA_{IgM} increase in a pair of serum samples in this study was taken as an indication of recent infection. Meurman et al. (6) found that anti-RSV IgM as detected by ELISA was present for 20 days to 2 to 3 months after the onset of disease. Popow-Kraup et al. (10) reported that in some cases IgM antibodies could be detected for up to 1 year after infection. Therefore, a single serum sample or a serum sample pair with a ΔA_{IgM} above the threshold value but without a significant increase, in combination with the clinical and epidemiological data on the onset of symptoms, can only be considered suggestive of a recent RSV infection. The incidence of RSV-specific IgM was significantly higher in infants than in children (13/15 versus 7/26; chi-square = 13.6, $P < 0.001$). The infants with a proven RSV infection in whom no IgM response was measured were all under 5 months of age. Meurman et al. (6) detected IgM after a primary RSV infection in all six children 1 to 2 years old but

in only five of the eight infants under 6 months old. Similarly, Welliver et al. (14) also found relatively few infants under 7 months of age with an IgM response. Watt et al. (13), however, reported an IgM immune response against purified RSV fusion protein in most infants under 6 months of age. Our ELISA showed absent or only weak IgM responses in children 1 to 7 years old. This result is in accordance with the results of Meurman et al. (6) but conflicts with those of Welliver et al. (14), for which we have no explanation.

In the control group, the ΔA_{IgA} was significantly greater in the children than in the infants, probably reflecting the presence of anti-RSV IgA for a prolonged period after an RSV infection. Therefore, although threshold values for ΔA_{IgA} in different age groups can be set, high levels cannot be used to identify a recent RSV infection. The incidence of an IgA response did not differ significantly between infants and children (13/15 versus 18/26; chi-square = 2.2, $P > 0.3$). Meurman et al. (6) concluded that for routine diagnostic use anti-RSV IgA determination has no advantage over IgG titer increase determination. In our study, however, an IgA immune response was found in 13 of 15 infants, whereas an IgG immune response could be found in only 3 infants. In accordance with our results, Murphy et al. (7) found that an immune response in the younger age group was detected most efficiently by a rise in IgA. As we found that of the 41 patients with an immune response detected with ELISA, 10 were found to have only an IgA response, we postulate that the detection of IgA is of diagnostic importance.

A single immunoglobulin class response was measured 5 times for IgG, 4 times for IgM, and 10 times for IgA. This finding results in increases in diagnostic yield for IgG of 14%, for IgM of 11%, and for IgA of 32%. In the patients without a proven RSV infection, mainly single immunoglobulin class responses were measured (12 of 14 cases with an immune response), of which IgA responses predominated (7 cases). This indicates that the measurement of all three immunoglobulin classes contributes to the diagnostic potential of this ELISA.

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